

IN THE CLAIMS

COMPLETE LISTING OF ALL CLAIMS, WITH MARKINGS AND STATUS IDENTIFIERS
(Currently amended claims showing deletions by ~~strike through~~ and additions by underlining)

This listing of claims will replace all prior versions and listings of the claims in the application.

Listing of Claims:

1-29. Cancelled.

30. (currently amended) A method for inducing an immune response in a warm-blooded animal comprising administering to the animal a composition comprising a bacterial cell, wherein

- (a) the bacterial cell comprises an expression gene that encodes an antigen, and an Environmentally Limited Viability System,
- (b) the antigen is introduced into the animal,
- (c) the bacterial cell is viable when in the animal and non-viable when outside of the animal, and
- (d) the Environmentally Limited Viability System comprises an essential gene that is under the control of an environmentally regulatable control sequence, wherein
 - (i) expression of the essential gene in the cell is essential to the viability of the cell,
 - (ii) the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal,
 - (iii) the essential gene is essential for metabolism, growth, cell wall integrity or cell membrane integrity of the bacterial cell, and
 - (iv) the essential gene is a copy of a native chromosomal gene wherein the chromosomal copy of said native gene is inoperable.

31. Cancelled.

32. (previously presented) The method of claim 30 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

33. (previously presented) The method of claim 30, wherein the composition is administered to mucousal surfaces of the animal.

34. Cancelled.

35. (currently amended) The method of claim 39, ~~wherein the essential gene, the lethal gene, or both is carried on an extrachromosomal vector, and~~ wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed when the cell is in the animal and not expressed when the cell is outside the animal, wherein the cell is a member of the *Enterobacteriaceae*.

36. (previously presented) The method of claim 30, wherein the bacterial cell is a member of the *Enterobacteriaceae*.

37. (previously presented) The method of claim 36, wherein the bacterial cell is an avirulent *Salmonella*.

38. (previously presented) The method of claim 37, wherein the avirulent *Salmonella* is an avirulent derivative of a pathogenic *Salmonella* that attaches to, invades and persists in the gut-associated lymphoid tissue or bronchial-associated lymphoid tissue.

39. (previously presented) The method of claim 30, wherein the system further comprises a lethal gene, wherein the expression of the lethal gene is lethal to the cell and the lethal gene is expressed when the cell is outside the animal but not when the cell is in the animal.

40. Cancelled.

41. (previously presented) The method of claim 30, wherein the essential gene encodes an enzyme which catalyzes the biosynthesis of the cell wall and its precursors.

42. (previously presented) The method of claim 41, wherein the essential gene encodes an enzyme which catalyzes a step in the biosynthesis of diaminopimelic acid (DAP).

43. (currently amended) The method of claim 42, wherein the essential gene is ~~an~~ the gene encoding β -aspartate semialdehyde dehydrogenase (*asd*) gene.

44. (currently amended) The method of claim 30, wherein the essential gene is selected from the group consisting of

genes encoding enzymes which catalyze steps in the biosynthesis of diaminopimelic acid (DAP) *dapA*, *dapB*, *dapC*, *dapD*, and *dapE*, the gene encoding alanine racemase (*dal*), the gene encoding D-alanyl D-alanine ligase (*ddl*), genes involved in fatty acid biosynthesis (*fab*), fatty acid degradation (*fad*), phospholipid synthesis (*pls*), a gene encoding a modification methylase, a gene encoding a DNA ligase, a gene encoding a DNA gyrase, and a gene encoding a phospholipase.

45. (previously presented) The method of claim 39, wherein the lethal gene is selected from the group consisting of a member of the *gef* family, a plasmid maintenance gene, a gene encoding a nuclease, a gene encoding a phospholipase, a gene encoding an endolysin, a gene encoding a holin, and a gene encoding the tRNA with a wrong codon.

46. (currently amended) The method of ~~claim 44~~, claim 45 wherein the lethal gene is the combination of bacteriophage P22 lysis genes 13 and 19.

47. (currently amended) The method of claim 35, wherein the replication gene is the gene encoding deoxyribonucleic acid polymerase I, (*polA*).

48. (previously presented) The method of claim 39, wherein the expression of the essential gene or the lethal gene is regulated by a trans regulatory element.

49. (previously presented) The method of claim 48, wherein the trans regulatory element is selected from the group consisting of a repressor, an antisense RNA, and an RNA polymerase.

50. (previously presented) The method of claim 39, wherein expression of the essential gene or the lethal gene is regulated by using promoters or regulatory elements that are regulated by temperature, or by other regulatory systems adapted to function in a temperature-dependent manner.

51. (previously presented) The method of claim 50, wherein the essential gene is regulated by being operatively linked to either

- (a) a *virB* promoter, wherein the bacterial cell further comprises a *virF* gene and promoter; or
- (b) a *virF* positive activator in combination with a promoter of *yopH* gene or a *yadR* gene.

52. Cancelled.

53. (currently amended) The method of claim ~~52~~51, wherein the essential gene is regulated by a bacteriophage lambda promoter left or right (λP_L or λP_R) P_L or a P_R promoter with a temperature sensitive bacteriophage lambda *cI857* repressor.

54. (previously presented) The method of claim 53, wherein the *cI857* repressor is operatively linked to a *P_{trc}* promoter.

55. (currently amended) The method of claim ~~52~~51, wherein expression of the lysis gene is regulated by a bacteriophage P22 P_R promoter operatively linked to a P22 *c2* gene, wherein the P22 *c2* gene is regulated by a P_L promoter, and wherein the cell further comprises a chromosomal *cI857* gene.

56. (currently amended) The method of claim 55, further comprising an essential gene operatively linked to a λP_L P_L promoter.

57. (previously presented) The method of claim 56, wherein the *cI857* repressor is inserted into an inactive chromosomal gene, wherein the inactive chromosomal gene is an inactive essential gene.

58. (previously presented) The method of claim 57, wherein the microbial cell is an avirulent *Salmonella*.

59. (previously presented) The method of claim 58, wherein the avirulent *Salmonella* is an avirulent derivative of a pathogenic *Salmonella* that attaches to, invades and persists in the gut-associated lymphoid tissue or bronchial-associated lymphoid tissue.

60. (previously presented) The method of claim 59, wherein the avirulent *Salmonella* further comprises an inactive gene selected from the group consisting of *cya*, *crp*, *phoP*, *phoQ*, *ompR*, *galE*, *cdt*, *htrA*, and a gene with a mutation that imposes a requirement for an aromatic amino acid or a vitamin.

61. (previously presented) The method of claim 57, wherein the extrachromosomal vector comprises pMEG-104.

62. (previously presented) The method of claim 61, wherein the extrachromosomal vector further comprises an expression gene.

63. (previously presented) The method of claim 62, wherein the expression gene encodes an antigen.

64. (previously presented) The method of claim 63, wherein the antigen is selected from the group consisting of a bacterial antigen, a viral antigen, a mycotic antigen, a parasitic antigen, a gamete specific antigen, and a tumor antigen.

65. (currently amended) The method of claim 30 ~~34~~, wherein the antigen is selected from a group consisting of a bacterial antigen, a viral antigen, a mycotic antigen, a parasitic antigen, a gamete specific antigen, and a tumor antigen.